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Review

Fungal (-like) biocontrol organisms in tomato disease control

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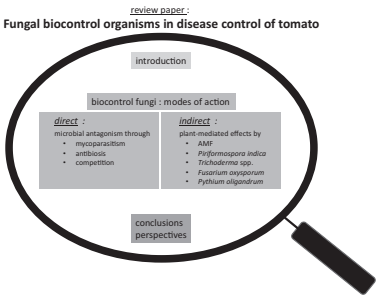
HIGHLIGHTS

- Several biocontrol-fungi (BCF) are under investigation for tomato disease control.
- These include AMF, *Piriformospora indica*, *Trichoderma* spp., *Fusarium oxysporum* and *Pythium oligandrum*.
- Tomato pathogens can be directly antagonized mainly by *Trichoderma* spp. and *P. oligandrum*.
- Induced systemic resistance (ISR) has been reported for all BCFs.
- Next-generation sequencing will improve our understanding of tripartite interactions.

GRAPHICAL ABSTRACT



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ABSTRACT

The worldwide important crop tomato is attacked by various pathogens, for which management is still primarily reliant on fungicides despite increasing concerns and constraints on their use. Other approaches are investigated, including the use of biocontrol organisms to manage tomato diseases. In this review we discuss and compare the interaction of major biocontrol fungi (BCF) with tomato, including the endophytic arbuscular mycorrhizal fungi and *Piriformospora indica*, the free-living opportunistic symbionts *Trichoderma* spp. and non-pathogenic *Fusarium oxysporum*, as well as the oomycete *Pythium oligandrum*. We cover recent advances that have been made in unraveling biocontrol modes of action against the most important tomato pathogens, encompassing direct effects of the BCF on pathogens and their indirect effects through the plant, with a main focus on induced systemic resistance. It is an exciting era for the study of biocontrol tripartite interactions, with the emergence of next-generation sequencing tools and the higher pace at which new genomes are being sequenced nowadays, as was recently also achieved for tomato. In addition, plant pathology and biocontrol research domains are increasingly reaching out to each other, because of the parallels that we are only beginning to discover between the interactions of beneficial and detrimental micro-organisms with a plant. Considering the enormous technological possibilities at hand today, this seems a timely opportunity to review the most recent advances in this field and to anticipate to what is ahead of us, discussing breakthroughs expected in our understanding of biocontrol interactions and remaining hurdles on the way to reach them.

1. Introduction

Tomato (*Solanum lycopersicum*) is one of the most important vegetables in the world, with global production in 2011 reaching almost 160 million tons (FAOSTAT 2013). Together with its global

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distribution and consumption it is therefore considered one of the most important horticultural crops worldwide. However, tomato production is hampered by various plant pathogens and pests, with fungal and oomycete pathogens in particular posing serious yield restraints (Foolad et al., 2008). Notorious tomato pathogens include the necrotrophic fungi *Botrytis cinerea* and *Alternaria solani*, the oomycete *Phytophthora infestans* and the vascular wilt fungus *Fusarium oxysporum* f. sp. lycopersici. *B. cinerea*, also known as grey mould, ranks second in the fungal plant pathogens top ten due to its broad host range and severe damage potential both pre- and post-harvest (Dean et al., 2012). The pathogen kills plant cells by producing phytotoxic compounds and cell wall degrading enzymes, after which the nutrients are extracted, typically causing soft rots followed by the appearance of grey conidia masses on aerial plant parts (Williamson et al., 2007). Like *B. cinerea*, the necrotroph *A. solani* which is the causal agent of tomato early blight can affect all above-ground plant parts. This pathogen causes one of the most common and destructive tomato diseases in areas with frequent rainfall or high humidity conditions, with symptoms including seedling damping off, stem and fruit rot and serious leaf blight up to complete defoliation of the plant (De La Noval et al., 2007). Another highly destructive foliar disease of tomato is caused by *P. infestans*. The disease, referred to as late blight, is characterized by leaf and stem necrosis, fruit rot and ultimately plant death. The pathogen has the potential to destroy tomato and potato crops within a matter of days after occurrence in the field, at any plant developmental stage (Foolad et al., 2008). The soil-borne *F. oxysporum*, ranking fifth in the fungal plant pathogens top ten, represents another threat to tomato production (Dean et al., 2012). Different formae speciales (f. sp.) exist for *F. oxysporum*, with f. sp. lycopersici infecting mainly tomato. The pathogenic strains penetrate plant roots and invade the vascular system, which typically leads to wilting of the aboveground parts and eventually plant death (Fravel et al., 2003).

The majority of the tomato cultivars grown in the field and in greenhouses for fresh tomato production carry dominant resistance genes effective against the most common races of *F. oxysporum* f. sp. lycopersici, thus providing a valuable addition to the management options for vascular wilt in tomato, which otherwise largely relies on chemical soil fumigation (Fravel et al., 2003). For the other main tomato pathogens discussed above, however, no commercial cultivars are available with adequate levels of resistance and management thus relies heavily on chemical control. For example, chemical control remains the most common method for reducing the devastating potential of *B. cinerea* to date (Blancard, 2012), with fungicides that specifically target this pathogen representing up to 10% of the global fungicide market (Dean et al., 2012). However, this pathogen is difficult to manage due to its various modes of attack and its capacity to bridge extended unfavourable periods as sclerotia in crop debris (Williamson et al., 2007). In addition, development of resistance to fungicides by *B. cinerea* has been reported multiple times (Leroux, 2007; Kretschmer et al., 2009). Benzimidazoles and dicarboximides, for example, have now largely been replaced due to widespread resistance, and an increasing number of *B. cinerea* strains is emerging with resistance against unrelated fungicides such as boscalid, fenhexamid, cyprodinil and iprodione (Leroch et al., 2011; Amiri et al., 2013). Fungicide resistance is also a problem in chemical control of late blight, with the regular appearance of highly aggressive strains that are resistant to the most commonly employed systemic fungicides such as the phenylamide metalaxyl (De La Noval et al., 2007). Apart from the high cost and risk of resistance development associated with fungicides, their use is also increasingly restricted due to their perceived harmful effects on both human health and environment. Combined with the fact that the cost of developing new chemical pesticides keeps on rising, the development of alternative management options is an urgent matter (Glare et al., 2012). In this context it is not surprising that beneficial micro-organisms receive great attention as a complementary approach in integrated disease management, with research focusing on the identification of new potential biocontrol organisms and their modes of action. This has already led to the exploitation of some biocontrol organisms (BCOs) in a commercial context, and the so-called biopesticide market is expected to grow in the years ahead (Glare et al., 2012).

In this review we will focus on fungi as BCOs, further termed biocontrol fungi (BCFs), and on their disease suppression potential in tomato, a crop that apart from its economic importance is also considered a model dicot for basic and applied research purposes. The recently sequenced genome (The Tomato Genome Consortium, 2012), will facilitate genome-wide molecular investigations of the interaction of the host plant with beneficial and detrimental micro-organisms. The most specialized BCFs are the well-studied arbuscular mycorrhizal fungi (AMF). Research on these obligate symbionts of the majority of all vascular plants has historically focused on their beneficial effects on plant growth and nutrition, while more recent research has investigated their bioprotective potential, mainly focusing on soil-borne pathogens (reviewed for example by Whipps, 2004, Trillas and Segarra, 2009). The other beneficial fungi that we will discuss also form endophytic associations with their host, but without a specialized interface for nutrient exchange as observed for AMF. In turn, these fungi can also exploit their saprophytic capabilities in order to survive in the soil. *Piriformospora indica* was isolated more recently from a spore of an arbuscular mycorrhizal fungus (Verma et al., 1998). In contrast to AMF, this mutualistic basidiomycete can be cultured axenically and has an exceptionally large plant host range encompassing both vascular plants (including the model plant *Arabidopsis thaliana*) and even mosses, thereby inducing protection against a variety of leaf and root pathogens (Qiang et al., 2012). The *P. indica* genome has recently been sequenced and annotated (Zuccaro et al., 2011), and will thus undoubtedly serve as a highly relevant model to study the molecular basis of the interaction between plants and beneficial fungal endophytes in the coming years.

In contrast to AMF and *P. indica* that are exclusively or predominantly found inside plant roots, other beneficial fungi are mainly found as free-living organisms in the soil. Hence their biocontrol effect was initially attributed solely to a direct interaction with plant pathogens, but more recently it was discovered that they are also opportunistic plant colonizers that can protect plants from pathogens through indirect mechanisms (Trillas and Segarra, 2009). *Trichoderma* spp. are undoubtedly the most investigated members of this category. Fungal strains belonging to this genus can be found in soils around the world and display a remarkable lifestyle range (Druzhinina et al., 2011). Apart from the relevance of certain strains for industrial applications, they also show great potential as BCFs conferring plant protection through various mechanisms against both below- and aboveground pathogens. *Trichoderma* spp. are the most successfully marketed BCFs in agriculture today, with more than 60% of the registered fungal biopesticides world-wide being based on this species (Mukherjee et al., 2012).

Other commonly found rhizosphere inhabitants are the saprophytic *F. oxysporum* strains. Although some important vascular wilt pathogens also belong to this species, other strains penetrate the roots without invading the vascular system and have been shown to protect plants against infection by their pathogenic counterparts (Alabouvette et al., 2009). A similar case is the soil borne, fungi-like oomycete *Pythium oligandrum*. Whereas the majority of the *Pythium* spp. are economically important plant pathogens, it is only in the last decade that the biocontrol potential of *P. oligandrum* has come to the attention of the scientific community (Benhamou et al., 2012).

These BCFs all share the ability to protect tomato plants from disease, which can be explained by various mechanisms. Direct interactions between BCFs and pathogens have been reported for most fungi and even were the focal point for research on opportunistic colonizers for many years. This so-called microbial antagonism encompasses parasitism, antibiosis and competition. However, since all BCFs are also capable of colonizing plant roots either in an obligate or an opportunistic manner, which in some cases also results in plant growth promotion, it seems logical that this is also linked to their biocontrol capacity. Indeed, an indirect biocontrol mechanism through induced systemic resistance (ISR) has also been reported or at least suggested for all BCFs involved. In the next sections we will systematically discuss the various modes of action proposed for these BCFs in the protection of tomato.

2. Microbial antagonism

2.1. Mycoparasitism

Since most BCFs are rhizosphere inhabitants, it is to be expected that they are well equipped to compete with other micro-organisms residing in the same ecological niche, or even to use them as a nutrient source. Mycoparasitism of plant pathogens is an important biocontrol trait of many *Trichoderma* spp. and also of *P. oligandrum*. In the case of *Trichoderma* spp., this is a multi-step process in which physical contact between the two micro-organisms is preceded by an early recognition stage. The BCF first constitutively produces and releases cell wall degrading enzymes (CWDEs) at low levels in an attempt to locate its prey. Upon detection of cell wall fragments from its target, directional growth towards the prey is induced together with a higher production of CWDEs, mostly chitinases, glucanases and proteases. *Trichoderma* spp. will then attach to their prey, coil around it and form appressoria to penetrate the hyphae, which are subsequently degraded through production of hydrolytic enzymes and secondary metabolites (Shoresh et al., 2010). Card et al. (2009) investigated the mycoparasitism process of *B. cinerea* by *Trichoderma atroviride* LU132 and found that hyphae of the pathogen rapidly collapsed and died within 4 days after the start of the BCF coiling around it. The recently published genome sequences of the highly mycoparasitic *Trichoderma virens* and *T. atroviride* indeed displayed an important enrichment in genes regulating the biosynthesis of antimicrobial secondary metabolites (see further) compared to that of the weakly mycoparasitic *Trichoderma reesei* (Mukherjee et al., 2012). Moreover, genes encoding for CWDEs such as β-1,3-glucanases appear over-represented in the *Trichoderma* genomes when compared with other related fungi (Kubicek et al., 2011). The mycoparasitic potential of *Trichoderma* spp. has been shown against a wide range of plant pathogens and this capacity seems to be widespread in the genus. For example, in a recent survey of more than 1 100 *Trichoderma* strains from 75 molecularly defined species, all strains were found to mycoparasitize *B. cinerea* (Druzhinina et al., 2011).

Horner et al. (2012) recently constructed the first *P. oligandrum* EST libraries, providing an initial overview on the biochemical cross-talk between this oomycete BCF and its prey. Transcripts encoding various CWDEs appeared to be induced in the BCF before physical contact with its prey, thus suggesting that the early recognition phase of *P. oligandrum* mycoparasitism resembles that of *Trichoderma*. The subsequent phases are also very similar, as *P. oligandrum* rapidly attaches to its prey, coils around it, forms papillae-like structures and finally penetrates through the production of hydrolytic enzymes. Active multiplication then takes place inside the hyphae of its prey, and the BCF is released again from dead hyphal cells (Benhamou et al., 1999). Like *Trichoderma*, *P. oligandrum* can directly attack a variety of fungal pathogens, but an interesting question that arises in this case is how the BCF distinguishes between self- and non-self cell wall degradation during mycoparasitism of closely related oomycete plant pathogens (Benhamou et al., 2012). Knowing that cellulose is a major component of oomycete cell walls, it has been suggested that the *P. oligandrum* cellulolytic enzymes that were found abundantly in the EST libraries, might function both in oomycete attack and in reconstruction of its own cell walls (Gruber and Seidl-Seiboth, 2012; Horner et al., 2012; Larroque et al., 2012).

2.2. Antibiosis

While *P. oligandrum* antagonism is probably mainly based on mycoparasitism, the BCF has also been reported to cause pathogen decay by the production of antimicrobial compounds without any physical contact between the micro-organisms, a process generally termed antibiosis. For example, the BCF was shown to kill the oomycete *Phytophthora megasperma* from a distance (Benhamou et al., 1999). This inhibitory activity was not accompanied by cell wall degradation, which is surprising because other members of the same genus were highly affected by the cellulolytic enzyme repertoire of *P. oligandrum* (Picard et al., 2000). The compounds responsible for this effect, however, have not been identified.

By contrast, the antibiosis potential of *Trichoderma* spp. has been better characterized. *Trichoderma* spp. are known to produce a wide array of secondary metabolites with antimicrobial activity against a diverse range of micro-organisms. The production of secondary metabolites is strain-dependent to a certain extent and includes both volatile and non-volatile antimicrobial compounds belonging to various chemical classes, including low-molecular weight non-polar compounds such as pyrones, butenolides, anthraquinones, trichothecenes and terpenoids, as well as non-ribosomal peptides such as peptaibols (Reino et al., 2008). Some compounds have been demonstrated to act synergistically in antagonism together with the CWDEs arsenal of the BCF. For example, Schirmbock et al. (1994) reported a synergistic effect of *Trichoderma harzianum* peptaibols trichorzianine A1 and B1 with chitinases and β-1,3-glucanases in inhibiting spore germination and hyphal elongation of *B. cinerea*. Moreover, as for the CWDEs, their abundant production is often triggered by the presence of a pathogen elicitor (Monte, 2001; Vinale et al., 2008), as was, for example shown for the peptaibol synthesis in *T. harzianum* in presence of *B. cinerea* cell walls (Schirmbock et al., 1994). One of the best-studied secondary metabolites is the pyrone 6-pentyl-2H-pyran-2-one or the 'coconut aroma' volatile compound, a metabolite that seems to be common to the *Trichoderma* genus and for which antifungal activity against *B. cinerea* has been demonstrated. Antifungal activity against several tomato pathogens has also been shown for purified butenolides and anthraquinones originating from various *Trichoderma* spp. (Liu et al., 2009; Vinale et al., 2009). Malmierca et al. (2012) recently also provided evidence for the role of trichothecenes in antibiosis of *B. cinerea* by showing that the antifungal activity of *Trichoderma* mutants defective in trichothecene formation was reduced. Finally, peptides such as the linear short-chain length peptaibols can cause membrane leakage due to their amphipathic nature that allows them to self-associate into oligomeric ion channels in the membranes subsequently leading to cell death. They were also shown to be induced by the presence of pathogen elicitors and to inhibit for example *B. cinerea* spore germination and hyphal elongation (Schirmbock et al., 1994; Chugh and Wallace, 2001). Cheng et al. (2011) recently reported that the extracellular protein L-amino acid oxidase secreted by *T. harzianum* ETS 323 was also involved in direct antagonism against *B. cinerea*, with hyphae of the pathogen showing an apoptosis-like

response, including ROS generation, DNA fragmentation and depolarization of mitochondrial membrane.

Instead of directly inhibiting the pathogen, the compounds produced by *Trichoderma* spp. can also disarm the pathogen by reducing its pathogenicity. For example, *T. harzianum* T39 produces cysteine proteases that significantly inhibit the activity of the *B. cinerea* arsenal of hydrolytic enzymes that the pathogen uses for infection, including various polygalacturonases, pectin methyl esterase, pectate lyase, chitinase, β-1,3-glucanase and cutinase (Zimand et al., 1996; Kapat et al., 1998; Elad and Kapat, 1999).

2.3. Competition

While mycoparasitism and antibiosis have been attributed only to *Trichoderma* spp. and *P. oligandrum*, competition as a potential biocontrol mechanism has been reported for the other BCFs as well. Competition for nutrients or for space and infection sites usually occurs between micro-organisms with the same physiological requirements in an ecological niche where resources might be limited. Competition for nutrients and especially for carbon is inherent to the soil environment, thus it comes as no surprise that this mechanism has been reported for arbuscular mycorrhizal fungi (AMF), *P. oligandrum* and *Trichoderma* spp., and is considered the primary mode of action for some non-pathogenic *F. oxysporum* strains (Alabouvette et al., 2009). For example, Larkin and Fravel (1999) attributed the biocontrol effect of *F. oxysporum* strain Fo47 to its strong capacity to compete for carbon with the tomato wilt pathogen *F. oxysporum* f. sp. *lycopersici*, while the biocontrol strain CS-20 seemed to suppress this disease through other mechanisms than competition. In the case of AMF, the transfer of assimilated carbon from a plant host to the fungus is estimated to range from 4% to 20% and thus it seems plausible that AMF compete with pathogens for this resource. Carbon competition has often been suggested as a mechanism through which AMF exert their biocontrol effect, but evidence in literature is scarce (Jung et al., 2012). Different AMF species have been reported to have a different carbon sink strength, and according to the hypothesis of carbon competition they should thus also exhibit different levels of biocontrol (Lerat et al., 2003). However, this claim does not seem to be supported by experimental evidence (Vierheilig et al., 2008). For example, *Glomus intraradices* could not exert a biocontrol effect on the pathogenic fungus *Phytophthora parasitica* despite its high carbon sink strength (Pozo et al., 2002). *Trichoderma* spp. have also been reported to compete for carbon. For example, only when glucose was limited, *B. cinerea* germ tube length was significantly reduced by *T. atroviride*. In the presence of other sugars such as sucrose and fructose, however, a significant inhibitory effect on germ tube length was observed at all tested concentrations (Card et al., 2009).

While competition for nutrients is closely linked to the soil and the rhizosphere, competition for infection sites usually occurs on or inside the plant roots. Intense colonization of the root surface can prevent access of the pathogen to its prefered infection sites, although there is some debate about this mechanism for non-pathogenic *F. oxysporum*. Biocontrol by the Fo47 strain against *Fusarium* wilt in tomato was only obtained when the BCF was applied in higher doses than the pathogen, which the authors ascribed to competition for infection sites since both strains normally colonize the same zones on the root surface (Fravel et al., 2003). It is difficult to exclude competition for nutrients in this case, however, since it is not clear whether specific infection sites exist for this pathogen and hyphae of both strains could still be observed at the same location on the root (Olivain et al., 2006). Competition for space or infection sites also seems plausible between AMF and root pathogens, since they both reside in the same part of the plant. Cordier et al. (1998) observed for example that arbuscule-containing tomato root cells and their neighboring cells were not invaded by the pathogenic fungus *P. parasitica*. This led to the assumption that the biocontrol effect of AMF might increase with the degree of colonization (Vierheilig et al., 2008), which was partly found true: a critical degree of colonization is considered as a prerequisite for biocontrol, typically characterized by the presence of arbuscules, although this is more likely related to indirect biocontrol mechanisms than to direct competition (Pozo and Azcon-Aguilar, 2007). The point of entry for a pathogen into the plant tissue comprises also wounds, senescing host tissues, or natural openings such as stomata and lenticels. These areas are generally nutrient-rich because of exudation of sugars and amino acids. Therefore, by colonizing wounds or senescing tissue *Trichoderma* spp. can also prevent infection (Mutawila et al., 2011).

3. Plant-mediated effects

Although direct antagonism is well described for most BCFs, in many cases the mechanisms highlighted in the previous section cannot explain the observed reduction in plant disease. For example, *T. virens* mutants impaired in antibiotic production (gliotoxin) or mycoparasitic potential were still able to reduce *Rhizoctonia solani* disease symptoms (Howell et al., 2000). In addition, in many cases soil-borne BCFs have also shown protection against disease when they were physically separated from the pathogen, either because it concerned infection by a leaf pathogen, or because the BCF and soil-borne pathogen were separated from each other in a so-called split-root set-up, in which the root system is distributed over two physically separated compartments. It is thus obvious that indirect, plant-mediated mechanisms also play a pivotal role in disease suppression. This systemic biocontrol effect can be closely linked with the capacity of the BCF to colonize plant roots. Indeed, even the beneficial fungi, both obligate and opportunistic symbionts, are initially recognized as root invaders by the plant because of the receptor-mediated perception of molecules that are often conserved among pathogenic and beneficial fungi (reviewed by Zamioudis and Pieterse, 2012). These molecules are termed microbe-associated molecular patterns (MAMPs) and the defense response induced in the plant upon MAMP-recognition by pattern recognition receptors (PRRs) is refered to as MAMP-triggered immunity (MTI), which forms the first line of defense of the plant attempting to limit further fungal invasion (Jones and Dangl, 2006). Remarkably, only very recently the existence of an MTI response in roots was described for the first time (Millet et al., 2010). In order to successfully establish an intricate symbiotic relationship with their plant host, the BCFs have developed various strategies to cope with the plant defense responses, either by attempting to reduce the induction of MTI or by actively suppressing it through the production of effector molecules (Zamioudis and Pieterse, 2012). This initial recognition phase of the BCF by the plant seems to prime the plant for a faster, stronger and often jasmonate (JA)-dependent defense response upon subsequent pathogen attack, a phenomenon that is termed induced systemic resistance (ISR). Traditionally, induced plant defenses are either categorized as systemic acquired resistance (SAR) or as ISR. SAR is typically defined as depending on the salicylic acid (SA) signaling pathway with the induction of the pathogenesis-related protein (PR) genes PR1, PR2 and PR2–5 (Durrant and Dong, 2004; Vlot et al., 2009). In contrast, ISR is thought to be SA-independent and regulated by the ethylene (ET) and jasmonate (JA) signaling pathway instead, not accompanied by major changes in PR-protein expression (Pieterse et al., 1996, 2009). However the overlap between SAR and ISR appears to be larger than originally expected as a consequence of crosstalk between the different hormonal pathways involved. This is, for example, demonstrated by

the NPR1 protein in *A. thaliana*, which plays a central role in SAR as a crucial transcriptional co-activator of SA-responsive genes, but is also required for ISR induced by *Pseudomonas fluorescens* WCS417 (Pieterse et al., 1998). In addition, the nature and composition of the ISR response seems to depend strongly on the tripartite plant-BCF-pathogen combination under study (Mathys et al., 2012). In the next sections we will discuss into detail the interaction of the five major BCFs with tomato and the implications for ISR. We will first describe the different colonization patterns of the BCFs, the recognition by their host and the induction of defense responses, in order to better understand the impact on ISR.

3.1. AMF

3.1.1. AMF – tomato colonization pattern

The obligate symbionts AMF obviously rely on plant roots for their survival and establish an intricate relationship with the plant host. Remarkably enough, AMF spore germination is independent of any plant signals, although a positive effect of root exudates has been reported (Tawaraya et al., 1996). Upon germination, hyphae with strong apical dominance protrude from the germination tube for a small distance. If no host signals are encountered, growth is stopped within a few days, hyphae will be sealed off and the cytoplasm will be retracted to the spore (Logi et al., 1998). The broad host range of AMF, together with this energy-saving mechanism and the possibility of repeated spore germinations seems enough to compensate for the host independent germination of this obligate symbiont (Giovannetti et al., 2002). The exploratory hyphal development from the asymbiotic phase changes drastically upon detection of plant-derived signals, resulting in increased hyphal growth and branching during which the AMF uses up all its reserves to increase the chance to actually encounter a root (Harrison, 2005). Only recently strigolactones have been identified as the plant-derived 'branching factors'. These compounds form a steep gradient in the rhizosphere due to their labile nature, and can thus be considered as good indicators for the proximity of plant roots (Parniske, 2008). The role of strigolactones in AMF symbiosis formation has been demonstrated by using strigolactone biosynthesis tomato knock-out mutants in a carotenoid cleavage dioxygenase (ccd8) or tomato plants expressing a cdd7 antisense construct, which in both cases resulted in significantly reduced mycorrhizal colonization (Koltai et al., 2010; Vogel et al., 2010). Another key element of the presymbiotic crosstalk is formed by the so-called Myc factors produced by AMF. These diffusible signals were recently identified as lipochitooligosaccharides (LCOs) and are probably ancestral to the more recent LCO-like rhizobial Nod factors (Maillet et al., 2011). The perception of these signaling compounds by the plant leads to the activation of the so-called symbiotic (SYM) pathway that is partially overlapping with the *Rhizobium*-legume symbiosis formation pathway (leading to nitrogen fixation) and facilitates subsequent root colonization (Oldroyd et al., 2013; Recorbet et al., 2013; Salvioli and Bonfante, 2013).

The physical contact between the fungus and the plant roots leads to morphological rearrangements in both partners characterized by differentiation of hyphae into appressoria and formation of a prepenetration apparatus in the epidermal cells to accommodate AMF penetration (Gianinazzi-Pearson et al., 2009). Penetration hyphae develop from the appressoria which will further colonize the root cortex, typically following either the *Arum* or *Paris* type growth pattern (Gao et al., 2004). In the *Arum* type, fungal hyphae grow intercellularly and branch off to form arbuscules, which are short intracellular branched hyphae with a treelike appearance, while in the *Paris* type plant cell wall penetration is much more frequent since hyphae grow directly from cell to cell, with the development of arbusculate coils in the colonized cortex cells (Kubota et al., 2005). It should be noted however that the plasma membrane of the plant is in no case penetrated and all fungal structures thus remain in the apoplast, resembling the haustoria formation in biotrophic pathogens (Nonomura et al., 2010). Arbuscules are pivotal for the symbiosis, forming the interface for nutrient exchange between the fungus and the plant (Vierheilig, 2004). Later in the symbiosis the fungus can also form storage structures designated as vesicles, and extend a network of hyphae outside the roots which is important for nutrient uptake (Giovannetti et al., 2002).

3.1.2. AMF – tomato recognition and interaction

Being obligate biotrophs, AMF share some similarities with biotrophic pathogens and transcriptional profiling of the plant response to both micro-organisms has indeed revealed significant overlap (Paszkowski et al., 2006). During the early stages of AMF symbiosis an MTI response is thus evoked in the plant, although the induction is typically only weak and transient. So far no MAMPs have been identified for AMF, but it is expected that such molecules are conserved among the biotrophic fungi regardless of their symbiotic or pathogenic nature (Paszkowski et al., 2006). An initial insight in this respect comes from the ectomycorrhizal fungi (EMF) that mainly establish a symbiosis with tree species and cover the roots with a dense mycelial mantle (Bonfante and Genre, 2010). In contrast with AMF, the genomes of two EMF species have already been assembled. The EMF *Laccaria bicolor* and the black truffle *Tuber melanosporum* apparently lack several gene families encoding CWDEs which could generate damage-associated molecular patterns (DAMPs) (Martin et al., 2008, 2010). These are plant-derived molecules typically released after plant cell wall degradation by micro-organisms, and their detection by the plant also induces defense responses. Thus, the absence of such genes points into the direction that mycorrhizal fungi attempt to avoid MTI stimulation as much as possible, which has also been observed in the case of biotrophic pathogens (Kamper et al., 2006; Baxter et al., 2010).

Global transcriptional changes in tomato upon colonization by the AMF *Funneliformis mosseae* (previously named *Glomus mosseae*) have been reported by Fiorilli et al. (2009). They performed microarray analysis of tomato roots and shoots as well as laser microdissection of arbusculate cells to study the cell-type expression profile of a subset of genes possibly involved in arbuscule development. Significant gene modulation was observed both in roots and shoots, with differentially expressed genes in the shoot being mainly down-regulated. The most responsive genes in both plant parts functioned in primary and secondary metabolism, defense and response to biotic and abiotic stimuli. Interestingly, many defense-related genes were down-regulated in the shoots, which was in contrast to what was found in other plants and could suggest greater susceptibility of mycorrhizal tomato plants to foliar pathogens, although the authors found the opposite in a *B. cinerea* infection test (Fiorilli et al., 2009). Results further suggested a role for auxin and abscisic acid (ABA) metabolism in arbuscule formation and functioning. The role of ABA in the establishment of a functional symbiosis was earlier also shown by Herrera-Medina et al. (2007), since tomato mutants deficient in ABA synthesis (sitiens) showed reduced AMF colonization, which could be restored by exogenous ABA application.

Another microarray analysis of tomato was performed by Lopez-Raez et al. (2010). The authors focused on transcriptional and hormonal profiles in tomato roots upon colonization by two AMF with different colonization patterns, namely *F. mosseae* and *G. intraradices*, to investigate to which extent the tomato response is dependent on the AMF species. Tomato roots colonized by both species showed a clear induction in oxo-phytodienoic acid (OPDA), a jasmonic acid (JA) precursor, while no differences in free JA levels

were observed. It is assumed that JA has a central role in the AMF symbiosis since an increase in free JA has been reported in many mycorrhizal plants (reviewed by Hause and Schaarschmidt, 2009), but experimental evidence for mycorrhizal tomato plants is controversial. Indeed, using tomato mutants affected in JA biosynthesis or signaling, both positive and negative regulatory roles of the JA pathway in AMF symbiosis have been found (Herrera-Medina et al., 2008; Tejeda-Sartorius et al., 2008). Lopez-Raez et al. (2010) therefore proposed OPDA as a main regulator of AMF symbiosis in tomato, also taking into account that in tomato higher levels of OPDA relative to JA accumulate upon wounding or pathogen attack (Miersch et al., 2008; Vicedo et al., 2009). The microarray analysis of Lopez-Raez et al. (2010) further also revealed the common induction of JA-biosynthesis and -signaling related genes such as LOXA, AOS1, AOS3, JAME and JAZ2 in mycorrhizal tomato roots. Several previously designated markers of AMF symbiosis were also found induced upon colonization by both species, including genes encoding a chitinase, glutathione S-transferase, β-1,3-glucanase, patatin and a PR10-like protein. The biosynthesis of oxylipins was also induced upon colonization by both AMF species. Two main branches of the oxylipin pathway exist in plants, with the 13-LOX branch leading to the biosynthesis of JA and its derivatives, whereas the 9-LOX branch results in the biosynthesis of ketols and 10-OPDA (Wasternack, 2007). The largely root-specific 9-LOX pathway was induced more strongly in the tomato–*F. mosseae* interaction, which the authors linked to the lower degree of colonization of this AMF species. In general, there was only 35% overlap between the transcriptional profiles of tomato roots in interaction with both species, with the specific pattern for *F. mosseae* indicating a more exhaustive control of the tomato plants over the *F. mosseae* colonization. Specific responses to *F. mosseae* colonization further included the induction of the isoleucine conjugate of JA (JA-Ile) and JA marker genes like proteinase inhibitors I and II. In addition, increased levels of salicylic acid (SA) and concomitant induction of PR1a were only observed in *F. mosseae* tomato roots.

All these data clearly show that AMF cannot avoid an initial MTI response, accompanied by transcriptional and hormonal changes in the plant in an attempt to limit invasion. However, the MTI response is transient since the AMF will use effectors to suppress this initial MTI response or to promote their symbiotic program. In line with the general observation that SA is a key regulator of plant defense against biotrophs (Glazebrook, 2005), SA seems to have a negative impact on AMF colonization. AMF therefore apparently try to suppress the SA-mediated defense in order to achieve a successful colonization (Jung et al., 2012). It is proposed that the establishment of the symbiotic program, which is activated in the plant upon perception of the mycorrhizal Myc factors, counteracts the MTI, although the precise mechanisms are yet to be elucidated (Zamioudis and Pieterse, 2012). The first AMF effector has only recently been described by Kloppholz et al. (2011). The SP7 effector of *G. intraradices* is induced upon contact with the host roots and this protein can cross the plant membrane to interact with the defense-related ethylene (ET)-responsive factor ERF19 in the plant nucleus to block the ERF19-mediated transcriptional program. The identification of SP7 as an AMF effector protein that interferes with ET signaling can be associated with recent reports highlighting the role of this hormone in MTI (Boutrot et al., 2010; Mersmann et al., 2010; Millet et al., 2010).

3.1.3. AMF – tomato-pathogen ISR

The intricate cross-talk between the AMF and its host with active modulation of defense responses is thus at the same time necessary to attain a successful colonization, as well as a prerequisite for ISR, which in the case of AMF is often refered to as mycorrhiza-induced resistance (MIR). Indeed, upon pathogen attack tomato plants with a fully established mycorrhizal symbiosis show a faster and stronger defense response, often dependent on a fully functional JA signaling pathway (Pozo and Azcon-Aguilar, 2007). For a comprehensive review summarizing the earlier work on MIR the reader is refered to Whipps (2004). MIR was shown for the first time by Cordier et al. (1998), who observed both local and systemic plant defense responses in mycorrhizal tomato against *P. parasitica* in a split-root set-up. The systemic defense response in non-mycorrhizal root parts of *G. mossae*-colonized tomato was characterized by the elicitation of host wall thickenings containing non-esterified pectins and the induction of the pathogenesis-related protein PR1a, as well as by the formation of callose-rich encasements around penetrating hyphae of the pathogen in order to halt its spreading (Cordier et al., 1998). Pozo et al. (2002) also reported a combination of local and systemic defense responses in the same three-way-interaction, with local induction of AMF-related new isoforms of the hydrolytic enzymes chitinase, chitosanase, β-1,3-glucanase and superoxide dismutase, involved in oxidative stress protection, while the lytic activity of some of the constitutive isoforms was altered in the systemic, non-mycorrhizal root parts. The authors observed MIR against *P. parasitica* when tomato plants were colonized by *F. mosseae*, but not by *G. intraradices*, which was concomitant with the induction of specific isoforms by *F. mosseae*. Since the authors more recently compared the transcriptional profiles in tomato roots after colonization with both AMF species, a role in *F. mosseae*-MIR was proposed for the highly induced 9-LOX oxylipin branch (Lopez-Raez et al., 2010), which is supported further by the report that resistance to *P. parasitica* in tobacco roots was dependent on the 9-LOX branch (Fammartino et al., 2007). In another study MIR was also observed against *B. cinerea* leaf infection of mycorrhizal tomato, which was correlated with the expression of the JA marker *Pin II*. Expression of the marker was induced after *B. cinerea* infection in both mycorrhizal and non-mycorrhizal plants, but the expression was markedly higher in mycorrhizal plants, clearly demonstrating the priming of JA-related defense responses in MIR (Pozo et al., 2010). Fiorilli et al. (2009) also found a decrease of *B. cinerea* disease symptoms in mycorrhizal tomato plants, although they had observed that many defense-related genes were down-regulated in the shoots. They concluded that the genes in question did not play a prominent role in the defense against this pathogen but rather hypothesized a link with ABA since genes involved in ABA catabolism were up-regulated in the shoots, and a decreased ABA content in leaves can contribute to increased *B. cinerea* tolerance (Fiorilli et al., 2011; Seifi et al., 2013).

3.2. P. indica

3.2.1. P. indica colonization pattern

Being only recently discovered as a BCF, so far no detailed studies have been undertaken of the tomato–*P. indica* interaction, although the disease suppressing potential of this BCF against *Verticillium dahliae* and *F. oxysporum* f. sp. *lycopersici* in tomato has already been reported (Fakhro et al., 2010; Sarma et al., 2011; Qiang et al., 2012). The colonization pattern has not yet been described in tomato, but the general pattern that emerges from observations on other plants seems to include two main phases: an initial biotrophic growth phase, followed by a cell-death dependent phase in which root cells are actively killed by the BCF (Jacobs et al., 2011). The crosstalk taking place between the fungus and its host plant preceding physical contact still has to be revealed for *P. indica*. Recent studies in *Arabidopsis* and barley indicated that upon contact with the roots, appressoria develop and the fungus will preferentially colonize the maturation zone of the roots, while it is hardly detected in the root meristematic and elongation zones (Deshmukh et al., 2006; Jacobs et al., 2011). Fungal growth is

mainly intercellular, however the root zone will become heavily infested by both inter-and intracellular hyphae and coiled branches. Plant cell death is then induced by the fungus, but seems to be restricted to the colonized cells alone. This phase appears to be a requirement for fungal proliferation, as dead rhizodermal and cortical cells become completely filled with chlamydospores. The fungus seems to achieve this by interfering with the host cell death machinery and not by the release of cytotoxic compounds (Shoresh et al., 2010). Thus the *P. indica* colonization process appears to be even more complex than that of AMF. The fact that this BCF, in contrast to AMF, is able to colonize *Arabidopsis* with a surprising colonization pattern has drawn the attention of the scientific community. An additional factor adding to the complexity of this symbiosis process is that endocellular bacteria seem to be intricately connected to *P. indica*, leading to the suggestion that they also play a role in the symbiosis and biocontrol potential of the fungus (Sharma et al., 2008).

3.2.2. P. indica – host recognition and interaction

Since *P. indica* has an exceptionally large host range, it can be inferred that it possesses an extraordinary capacity to deal with the defense responses of its host and possibly targets conserved recognition and signaling pathways (Lahrmann and Zuccaro, 2012). So far nothing is known about *P. indica* MAMPs or its ability to release DAMPs from its host, but the question whether *P. indica* evades or suppresses the MTI was recently addressed by Jacobs et al. (2011) in *Arabidopsis*. In a first series of experiments they treated *P. indica*-colonized roots with several well-known MAMPs including the bacterial flagellin (Flg22) and a fungal chitin octamer (N-acetylchitooctaose; Glc8), which are generally known to trigger an MTI response in the plant (Gomez-Gomez et al., 1999; Zipfel et al., 2004; Miya et al., 2007). Surprisingly, *P. indica*-colonized roots were almost completely nonresponsive to these MAMPs both in terms of marker gene expression and callose deposition, clearly indicating the capacity of the BCF to actively suppress the MTI response. Treatment of seedlings with the abovementioned standard MAMPs before inoculation with *P. indica* significantly reduced subsequent colonization, thus indicating that the root MTI is indeed also effective against this BCF. At present it can thus be concluded that *P. indica* most probably contains MAMPs that are able to elicit an MTI response in the plant, which the BCF can then apparently actively suppress in a highly effective manner (Jacobs et al., 2011). Based on their presence in the recently annotated *P. indica* genome, fungal lectins and small secreted proteins (SSPs) are highlighted as candidate effectors to suppress the MTI response in early colonization steps (Lahrmann and Zuccaro, 2012).

The transient nature of the MTI response during *P. indica* colonization has for example been demonstrated by Schäfer et al. (2007) in barley, in which they noticed the transient up-regulation of the defense-related markers PR1b, PR2 and PR5 only at the early stages of the colonization. Even the cell-death phase of the colonization is not accompanied by necrosis formation or visible cell wall reinforcements, as opposed to the strong defense response that can be induced by necrotizing pathogens (Deshmukh et al., 2006). Early plant responses have been reported to include production of reactive oxygen species (ROS), while later on in the colonization defense responses include SA-mediated responses and production of indole glucosinolates (Jacobs et al., 2011). Hence *Arabidopsis* mutants impaired in SA or glucosinolate-associated defenses were found to be more susceptible to *P. indica* colonization (Sherameti et al., 2008; Jacobs et al., 2011). Similarly, mutants lacking monodehydroascorbate reductase 2 (MDAR2) and dehydroascorbate reductase 5 (DHAR5), involved in antioxidant reduction, were hypersusceptible to *P. indica* (Vadassery et al., 2009). A central role in the *P. indica* plant interaction is proposed for JA (Qiang et al., 2012). *P. indica* colonization was shown to up-regulate markers for JA biosynthesis and signaling in the roots and as such suppression of the MTI response is thought to involve the JA signaling pathway and more specifically JAR1 and MYC2, since the fungus could not suppress MTI in the corresponding *Arabidopsis* mutants *jar1* and *myc2* (Schäfer et al., 2009; Jacobs et al., 2011). However, the involvement of other plant hormones has been reported as well. Transcriptional analysis of barley to *P. indica* colonization revealed that the biotrophic colonization phase was accompanied by induction of genes involved in ABA and auxin pathways, while the cell-death phase was associated with increase in gibberellic acid (GA) and brassinosteroids (Schäfer et al., 2009). Barley mutants impaired in gibberellic acid (GA) showed elevated root defense responses and reduced root colonization (Schäfer et al., 2009), while a higher colonization was observed the *Arabidopsis* mutants *etr1*, *ein2* and *ein3* related to ET-signaling (Camehl et al., 2010).

3.2.3. P. indica – host-pathogen ISR

Research on *P. indica* has largely focused on interactions with root pathogens, without any reported direct effect of the BCO on the pathogen, but ISR has also been clearly observed against leaf pathogens (Stein et al., 2008; Waller et al., 2008; Franken, 2012). For example, powdery mildew leaf infection of *P. indica*-colonized barley plants, induced a subset of defense-related genes in a faster and stronger manner than in non-colonized plants (Molitor et al., 2011). In tomato, disease-suppressive effects against *V. dahliae* and *F. oxysporum* have been reported (Fakhro et al., 2010; Sarma et al., 2011; Qiang et al., 2012). With the recent in-depth sequencing of the tomato genome, molecular studies focusing on the tomato–*P. indica* interaction are expected to emerge soon. A question that remains is to what extent the endobacteria that live in association with *P. indica* have a role in its biocontrol effect. Remarkably enough the fungus could not yet be cured from its bacterial occupiers, which belong to the genera *Rhizobium*, *Acinetobacter*, *Paenibacillus* or *Rhodococcus* (Sharma et al., 2008). By contrast, *Rhizobium radiobacter* could be isolated from *P. indica* and propagated axenically. Surprisingly, when directly applied to barley plants, the bacterium was able to induce an ISR response to powdery mildew (Sharma et al., 2008), thus it seems that both partners in the fungal-bacterial association contribute to ISR.

3.3. Trichoderma

3.3.1. Trichoderma colonization pattern

Trichoderma spp. are common inhabitants of the soil and recently it was reported that their endophytic behavior has originated more recently in evolution compared to their mycoparasitic lifestyle (Kubicek et al., 2011). It is speculated that the presence of fungal prey and plant–root derived nutrients has attracted the *Trichoderma* ancestors to the plant rhizosphere (Druzhinina et al., 2011). In this context it is interesting to note that *Trichoderma* spp. were able to employ the extraradical mycelium of AMF as a gateway to enter potato roots, a trait that might have facilitated the evolution of endophytism (De Jaeger et al., 2010). Some *Trichoderma* strains can colonize only local sites on roots, while the so-called rhizosphere-competent strains are able to establish a long-lasting colonization with their host (Harman et al., 2004). The general colonization pattern of *Trichoderma* spp. starts with hyphae coiling around the roots and forming appressoria-like structures to penetrate the root cortex (Yedidia et al., 1999). It was shown that even before colonization the fungus can produce auxins to promote root growth, which facilitates colonization due to the increased root surface area (Contreras-Cornejo et al., 2009). The fungus then grows intercellularly in the root epidermis and the cortex, although usually limited to the outer cortical layers because of restrictions imposed by the plant (Yedidia et al., 1999).

Chacon et al. (2007) studied the colonization pattern of *T. harzianum* CECT 2413 in tomato in detail by confocal microscopy with GFP-fused *T. harzianum*. When either hydroponically or soil-grown tomato plants were inoculated with conidia of this strain, a similar colonization pattern was observed. First a dense hyphal network covering the entire main root could be observed, with hyphae attaching to the root and settling in the grooves between epidermal cells which led to a subtle disorganization of this cell layer. This is followed by colonization of the epidermis and cortex, with intercellular hyphal growth characterized by the formation of swollen, papilla-like hyphal tips, which seemed to be induced by the plant as they were detected in much lower levels in hyphae grown in absence of tomato roots. The *Trichoderma* strain then undergoes further morphological changes that have thus far only been observed in this specific interaction, switching to a yeast-like cell type that remained attached to the root surface (Chacon et al., 2007). In the colonization process of tomato by *Trichoderma hamatum* T382 these structures could not be observed (Yang et al., unpublished results). Remarkably, some *Trichoderma* spp. have been isolated from aerial plant parts, although even in that case they were afterwards able to colonize roots (Bae et al., 2011).

3.3.2. Trichoderma – tomato recognition and interaction

Trichoderma colonization clearly does not remain unnoticed to the plant, that restricts the internal fungal growth by callose deposition in the surrounding plant cells and production of phenolic compounds (Yedidia et al., 1999). *Trichoderma* spp. release various compounds that are believed to act as MAMPs and thus contribute to this MTI response. These include proteins with enzymatic activity or hydrophobin-like properties and various secondary metabolites originally identified for their direct antifungal activity such as peptaibols (reviewed by Hermosa et al., 2012). The first recognized MAMP was a xylanase from *Trichoderma viride*, more specifically the ET-inducing xylanase (Xyn2 or Eix). This is a potent elicitor of various responses in tomato and tobacco, including a hypersensitive response (Rotblat et al., 2002), for which the corresponding plant PRR receptor (Eix2) has even been identified. The catalytic activity of Eix is not required for eliciting defense responses in the plant, indicating that the enzyme itself acts as a MAMP and binds to the receptor (Ron and Avni, 2004). Another MAMP was identified from *T. harzianum* CECT 2413 as a cell-wall degrading endopolygalacturonase ThPG1 (Moran-Diez et al., 2009). This MAMP was also shown to be necessary for active colonization of tomato, since *Thpg1*-silenced *Trichoderma* transformants displayed a reduced colonization capacity. Vinale et al. (2008) reported that certain secondary metabolites produced by various *Trichoderma* strains such as 6-Pentyl-α-pyrone, harzianolide and harzianopyridon exerted a direct antimicrobial effect at high doses, but act as MAMPs at low concentrations, thus activating MTI and regulating plant growth in tomato. As for the other BCFs, additional compounds that can trigger plant defense are oligosaccharides and low-molecular-weight compounds released from fungal or plant cell walls as a consequence of the activity of *Trichoderma* enzymes (DAMPs) (Woo and Lorito, 2007). Chitin oligosaccharides have in this respect been shown to be elicitors of MTI, as they are bound by the lysine motif LysM receptor-like kinase CERK1 (Kishimoto et al., 2010). Thus, the wide range of chitinases secreted by *Trichoderma* spp. for direct antagonism can indirectly also evoke an MTI response.

The changes induced in the plant upon *Trichoderma* colonization have already been studied in various plants at transcriptome, proteome or metabolite level and include in general extensive alterations in defense-related functions, enhanced photosynthesis efficiency under stress, oxidative stress protection, activation of cell wall metabolism, lytic enzymes and production of phytoalexins (reviewed by Shoresh et al., 2010). The first microarray analysis of the tomato–*T. hamatum* T382 interaction was carried out by Alfano et al. (2007), focusing on the differential gene expression in leaves after *Trichoderma* colonization of roots. Most of the differentially expressed genes had functions associated with biotic or abiotic stress, with a notably strong induction of the SA marker PR5. Other SA markers, however, were not induced even though they were represented on the microarray. The expression of genes involved in JA and ET signaling pathways such as *Lox1*, *Pal1*, *ETR1* and *CTR1* was not altered, although in general a central role has again been ascribed to the JA signaling pathway for plant–*Trichoderma* interactions. This is confirmed in our recent results obtained with tomato signaling mutants in interaction with the same T382 strain, which indicate that the ISR triggered by *T. hamatum* T382 in tomato is JA biosynthesis-dependent (Yang et al., 2013). Alfano et al. (2007) also found plant cell wall metabolism to be altered in the leaves, as shown by the induction of extensins and extensin-like proteins (Alfano et al. 2007). Extensins are not only involved in plant cell wall changes, but also in induction of plant defense and in this respect they have also been shown to be involved in rhizobia-mediated ISR (Shoresh et al., 2010). Tucci et al. (2011) investigated the interaction of *T. atroviride* P1 and *T. harzianum* T22 with various wild and cultivated tomato lines. They performed expression analysis by qRT-PCR of markers for SA (*PR1b1* and *PR-P2*) and JA (*PINI*, *PINII*, *TomLoxA* and *TomloxC*) pathways. Although the genetic variability among the tomato lines greatly affected the outcome of the interaction, the SA-mediated defense response was generally up-regulated by the *Trichoderma* strains as indicated by the induction of the SA markers, whereas JA-related genes seemed less responsive. The authors showed in addition that the up-regulation of SA markers was relatively long-lasting in the most responsive tomato genotypes, since induction of particularly *PR1b1* and *PR-P2* was still observed after 60 days. Thus, it seems that the long-term response to *Trichoderma* spp. in tomato involves SA signaling, at least in absence of pathogen infection (Tucci et al., 2011). Malmierca et al. (2012) investigated the response of the same SA and JA marker genes in tomato leaves after root colonization by *Trichoderma arundinaceum*, a species that had not yet been described as a BCO before, and found that *T. arundinaceum* induced expression of both SA (*PR1b1*) and JA markers (*PINI* and *PINII*). Since this strain is also known to produce the trichothecene harzianum A (HA), the authors constructed a mutant with aberrant HA biosynthesis to evaluate the role of HA in biocontrol. The *tri4* mutant, that does not produce HA, did not only display reduced direct antifungal activity against *B. cinerea* and *R. solani*, but also reduced induction of the JA and SA markers as compared to the wild type strain. It thus seems that HA can be considered as another MAMP for *Trichoderma* spp.

3.3.3. Trichoderma – tomato-pathogen ISR

One of the first reports on *Trichoderma*-ISR in tomato was by De Meyer et al. (1998). Although *T. harzianum* T39 was present only on the roots, a significant reduction in *B. cinerea* symptoms was observed on the leaves. Since then many studies have focused on the *Trichoderma*–plant interaction, and this earlier work has been reviewed by Benitez et al., 2004. However to date few examples exist in literature where the tripartite ISR interaction has been examined at the molecular level. Alfano et al. (2007) observed an ISR effect of *T. hamatum* T382 against tomato bacterial spot caused by *Xanthomonas euvesicatoria* (syn. *Xanthomonas campestris* pv. vesicatoria), however they only performed a microarray analysis of the *Trichoderma*–plant interaction just before pathogen inoculation. Tucci et al. (2011) looked at the induction of SA and JA markers after infection by *B. cinerea* and noted a clear ISR effect of *T. atroviride* P1 and *T. harzianum* T22 for some tomato genotypes, however not for all lines. While in the absence of a pathogen the *Trichoderma* strains

mainly induced the SA markers, upon pathogen infection the SA-dependent gene expression seemed to be mitigated by the BCFs and the JA-mediated response was promoted. Indeed, the expression of the JA markers *PINI*, *PINII*, *TomLoxA* and *TomLoxC* was enhanced in the tripartite interaction, with T22 causing the most consistent repression of SA and stimulation of JA markers across the different tomato genotypes. Chowdappa et al. (2013) infected tomato plants with *A. solani* or *P. infestans* and found that disease symptoms were significantly reduced when plants were colonized by a *T. harzianum* strain. The ISR response was accompanied by increased IAA and GA levels, as well as increased activity of polyphenol oxidase, peroxidase and superoxide dismutase. These increased enzyme activities point towards increased oxidative stress protection in *Trichoderma*-colonized tomato roots, which was previously also shown in tripartite interactions in *Arabidopsis* (Mathys et al., 2012). Vinale et al. (2008) demonstrated that ISR can also be induced by the secondary metabolites secreted by *Trichoderma* spp. A reduction of *B. cinerea* disease symptoms on tomato leaves was observed after treatment of the seedlings with the purified secondary metabolites, which was concomitant with an induction of several defense-related genes like chitinase, PR1 and endochitinase. Finally, it is interesting to note that the ISR effect has been demonstrated to be long-lasting, at least for the commercialized *T. harzianum* T22 strain. In field trials with tomato, disease caused by natural infection by the early blight pathogen *A. solani* was significantly reduced by T22 root drench treatment at transplanting, more than 100 days earlier (Harman et al., 2004).

3.4. F. oxysporum

3.4.1. F. oxysporum colonization pattern

The colonization pattern of the protective strain Fo47 has been described in several plant species, including tomato, by transforming the strain with different reporter genes (Bolwerk et al., 2005; Olivain et al., 2006; Nahalkova et al., 2008). Similar to *Trichoderma* spp., the fungus typically intensively colonizes the tomato root surface. Hyphae have then been observed to penetrate root hairs or the epidermis either inter- or intracellularly, however there are relatively few penetration sites compared to the high hyphal density on the root surface. Appressoria formation as such has not been found, but specialized penetration structures do seem to develop as hyphal swellings at the penetration sites on tomato roots. The fungus does not seem to have a preference for certain root zones as penetration sites are found on both young and mature root parts (Alabouvette et al., 2009). Differences in the colonization pattern of tomato roots have been observed depending on the experimental system used. For example, both *in vitro* and in hydroponics whole roots become heavily colonized with a uniform distribution of conidia on the root surface and intensive colonization at the root apices where exudates are abundant, while in soil the roots seem to outgrow the germination conidia and apices are thus not colonized. In addition, it was observed that germ tubes in the soil do not show a particular directed growth toward the root surface (Olivain et al., 2006; Nahalkova et al., 2008). Fungal growth inside the root was mainly restricted to the outer layers of the root, including the epidermis, hypodermis and rarely the outer cortex layers, due to the defense reactions induced in the plant. Only in a few cases hyphae were observed in the inner cortex which the authors linked to the high inoculum pressure used in those particular experiments. In contrast to the pathogenic *F. oxysporum* strains, the beneficial, non-pathogenic strains thus never reach the stele (Alabouvette et al., 2009).

3.4.2. F. oxysporum – tomato recognition and interaction

Defense reactions are clearly induced in the tomato plant roots upon colonization by the non-pathogenic *F. oxysporum* strains. Structural barriers are created by thickening and coiling of the cell walls around the penetrating fungus, accompanied by cell wall appositions, intercellular plugging and intracellular osmiophilic deposits (Benhamou and Garand, 2001). The invading Fo47 strain was even reported to suffer from plasma membrane breakdown and cytoplasm disorganization, and a local hypersensitive response appears to take place (Olivain et al., 2003). The non-pathogenic strains thus clearly elicit an MTI response in the plant, which is stronger than in the case of pathogenic strains that consequently succeed in reaching the vascular cylinder (Fravel et al., 2003). It is not known whether the differences in plant response upon colonization by a non-pathogenic or a pathogenic strain are due to differences in MAMPs, or due to the existence of specific effectors that modulate the defense response. Secondary metabolites produced by *F. oxysporum* strains are candidate MAMPs, including fusaric acid, necrosis-and-ethylene producing protein Nep1 and tomatinase, which have all been shown to elicit plant defense responses at low concentrations (Ito et al., 2004; Bae et al., 2006; Bouizgarne et al., 2006). However so far it has not been investigated whether they are specific for pathogenic or non-pathogenic strains and thus whether they could be implicated in the priming of ISR.

Tomato plants colonized by non-pathogenic *F. oxysporum* were reported to have higher root activity of chitinase, β-1,-3-glucanase and β-1,4-glucosidase compared to non-colonized plants (Tamietti et al., 1993; Fuchs et al., 1997), while in leaves an enhanced accumulation of PR1 and chitinases was observed (Duijff et al., 1998). The accumulation of ferulic, caffeic and vanillic acid was equally found increased in the leaves of tomato plants colonized by a non-pathogenic strain as compared to non-colonized controls (Panina et al., 2007). However when these responses are compared to those induced by pathogenic *F. oxysporum* strains, the plant response is generally reported to be lower in the beneficial interaction, although global transcriptomic studies are still lacking. Recorbet et al. (1998) reported for example a lower activity of chitinase and β-1,-3-glucanase in roots colonized by the non-pathogenic strains as compared with the pathogenic strain. Similarly, Aime et al. (2008) found a lower expression of β-1,-3-glucanases, chitinases and PR1a in tomato roots after inoculation with the non-pathogenic strain. More recently, the same authors investigated the expression of several other defense-related genes in tomato roots after inoculation with the non-pathogenic Fo47 and pathogenic Fol8 strain (Aimé et al., 2013). Enhanced expression was observed for a basic intracellular chitinase, a basic intracellular β-1,-3-glucanase and a lipoxygenase, which was always higher in case of Fol8, thus pointing to a possible role for both SA and JA-mediated mechanisms.

It must be noted, however, that the borderline between a beneficial and pathogenic strain is very thin in the case of *F. oxysporum*. Considering the narrow host specificity of the pathogenic strains, the absence of pathogenicity in a certain plant species could simply be a consequence of incompatibility with a non-host plant, which may suffice to elicit the defense responses in the plant and protect it against later attacks. So-called non-pathogenic strains should thus actually be tested on a very wide range of plants before they can actually be classified as such (L'Haridon et al., 2011).

3.4.3. F. oxysporum – tomato-pathogen ISR

For a summary on the earlier work of ISR by non-pathogenic *F. oxysporum*, the reader is refered to the review by Fravel et al. (2003). For example, non-pathogenic *F. oxysporum* isolates such as CS-20 have been shown to be very effective in reducing *Fusarium* wilt disease of tomato under a broad range of environmental conditions (Larkin and Fravel, 2002). A certain degree of root colonization seems to be a prerequisite for the induction of ISR, since mutants of non-pathogenic strains that were affected in

their ability to penetrate the tomato roots had lost their protective effect against the pathogenic strain (Di Pietro et al., 2001; Duyvesteijn et al., 2005; Alabouvette et al., 2009). Since the protective effect of the non-pathogenic strains has in most cases been investigated against pathogenic strains of the same species, ISR has mainly been demonstrated by the use of split-root systems (Fuchs et al., 1997; Duijff et al., 1998; Larkin and Fravel, 1999; Aime et al., 2008). A systemic effect in the leaves was observed by Kavroulakis et al. (2007), who reported that the non-pathogenic *Fusarium solani* Fs-K strain was able to colonize tomato roots and subsequently protect the plants against the foliar pathogen *Septoria lycopersici*. They employed mutant tomato lines to determine the role of ET (Never ripe and epinastic) and JA (def1) in the ISR response and found that the ET signaling pathway seemed to be required for the ISR effect. Evidence for a primed plant response was recently provided by Aimé et al. (2013). The authors set up an *in vitro* experimental system enabling accurate tomato root inoculation with either non-pathogenic Fo47 or pathogenic Fol8, in such a way that the fungi could be kept physically separated from each other. The use of specific markers allowed them to quantify fungal biomass of each strain in the root, and clearly indicated that Fol8 growth was reduced when roots had been pre-inoculated by Fo47. This was concomitant with a higher increase in an acidic extracellular chitinase, acidic extracellular β-1,-3-glucanase and PR1a in roots pre-inoculated with Fo47, which was not observed when either strain was present alone in the roots and could thus play a role in the observed disease suppression (Aimé et al., 2013).

3.5. P. oligandrum

3.5.1. P. oligandrum colonization pattern

The thin borderline between beneficial and pathogenic microorganisms is also well exemplified by the beneficial oomycete *P. oligandrum*. This BCF displays an unusual colonization pattern which consists of a rapid colonization phase of the whole root in a way similar to pathogenic oomycetes, while induction of plant responses will lead to its subsequent degeneration without causing any damage to the root tissue (Rey et al., 1998). Cytological investigations of *P. oligandrum*-inoculated tomato roots revealed that the BCF proliferates at the root surface and readily penetrates the epidermis throughout the root. Once inside the root *P. oligandrum* will spread to all root tissues, even including the vascular stele. This growth phase is soon followed by the sudden degradation of the oomycete, with changes in the hyphal structures being observed as early as 14 h after inoculation. The oomycete cells will then gradually degenerate and finally become empty structures, while typical oogonia are formed (Le Floch et al., 2005).

3.5.2. P. oligandrum – tomato recognition and interaction

The fast and extensive MTI response of the plant indicates that *P. oligandrum* MAMPs are well perceived by the plant. At least two MAMPs have already been characterized so far. The first described MAMP was oligandrin, a 10 kDa elicitin-like protein (Picard et al., 2000). Application of oligandrin was reported to induce resistance in various plants including tomato against pathogens like *P. parasitica*, *F. oxysporum* and *B. cinerea* (Benhamou and Garand, 2001). The nature of the elicited response in tomato seemed to vary according to the challenging pathogen. For example, upon oligandrin treatment a strong stimulation of the phenylpropanoid and terpenoid pathways was found in the tomato–*P. parasitica* interaction, while in the tomato–*F. oxysporum* interaction a massive deposition of cell wall appositions at the sites of pathogen penetration was observed, together with the production of various antimicrobial compounds (Picard et al., 2000). *P. oligandrum* cell wall glycoproteins (CWP), termed POD-1 and POD-2, are also potent elicitors of the MTI response (Takenaka et al., 2006). These MAMPs have a conserved region with six cysteine residues similar to that found in oligandrin and are also classified as elicitin-like, based on their ability to elicit plant defense responses without the induction of a typical hypersensitive response. The same authors recently reported that POD-1 and POD-2 need to form a specific 3D structure in order to display their elicitor capacity (Takenaka et al., 2011). Masunaka et al. (2010) demonstrated that both MAMPs were found only in the *P. oligandrum* genome and not in other *Pythium* spp. Similar to oligandrin, application of the CWP fractions either as a root drench or foliar spray was reported to protect plants against various pathogens, with the induction of the phenylpropanoid pathway, PR proteins and cell-wall bound phenolic compounds (Takenaka et al., 2003; Takenaka and Tamagake, 2009).

The response of the plant upon *P. oligandrum* colonization involved the formation of cell wall appositions as well as the induction of the phenylpropanoid and terpenoid pathways, which was well correlated with the structural changes in the *P. oligandrum* hyphae (Le Floch et al., 2005). The degeneration of the oomycete by the plant clearly indicate that it does not have the ability to avoid or suppress the MTI response, unlike the other BCFs. The signaling pathways involved in this reaction have not yet been investigated, and the only information comes from studies performed with the *P. oligandrum* CWP. Treatment of tomato plants with CWP did not induce SA accumulation or induction of the SA-marker PR1 (Hase et al., 2008). Furthermore, no up-regulation of SA-related genes was observed in a gene expression analysis of CWP-treated tomato with cDNA arrays (Takahashi et al., 2006).

3.5.3. P. oligandrum – tomato-pathogen ISR

P. oligandrum has been shown to protect tomato plants against several fungal and bacterial pathogens by ISR (Benhamou et al., 1997; Le Floch et al., 2003). Tomato plants that have been pretreated by *P. oligandrum* typically react to subsequent pathogen infection by a combination of physical and chemical responses to limit penetration and spread of the pathogen. For example, tomato plants pretreated with *P. oligandrum* and then infected by *F. oxysporum* f. sp. lycopersici displayed enhanced callose deposition that succeeded in preventing the pathogen from entering the vascular cylinder. In addition, enhanced phytoalexin production and PR protein synthesis was observed (Benhamou et al., 1997). Tomato infection by the bacterial wilt pathogen *Ralstonia solanacearum* was also halted by the rapid formation of structural barriers (Masunaka et al., 2009). Hase et al. (2008) showed that the ISR response against *R. solanacearum* was not compromised in CWP-treated transgenic tomato plants expressing the nahG gene encoding an hydroxylase that degrades SA. On the contrary, the ISR effect was abolished in the jai1 mutant, with an impaired JA signaling pathway, thus indicating the importance of the JA-dependent signaling pathway in this case. An ISR effect was also observed against *B. cinerea* infection of the aboveground tomato plant parts, which was accompanied by the induction of β-1,-3-glucanase (Le Floch et al., 2003). Finally, Le Floch et al. (2009) recently found the ISR capacity of *P. oligandrum* equally effective as that of *T. harzianum* and *F. oxysporum* Fo47 in controlling *B. cinerea* infection in tomato, which was accompanied by an induction of chitinase PR3 protein.

3.6. Other plant-mediated effects

Besides the induction of ISR, other indirect, plant-mediated effects can also contribute to increased disease tolerance. For most of the above-mentioned BCFs (AMF, *P. indica*, *Trichoderma* spp. and *P. oligandrum*) the ability to increase nutrient uptake and promote plant growth has been reported. The nutrient exchange between

the BCF and its host has been best documented for AMF, forming typical structures in the plant for this purpose, a specialization which has not been observed for the other BCFs. Indeed, while nutrient transporters have also constituted a central role in AMF research for many years, the nutritional strategies of the other BCFs as well as their nutrient transfer mechanisms are still largely unknown (Lahrmann and Zuccaro, 2012). Since long AMF have been known to increase the uptake of water and mineral nutrients for their host plant, especially of phosphate, while in turn they receive photosynthetic carbon from their host (Gianinazzi et al., 2010). Together with the increased protection against abiotic stresses, this can also render the plant more tolerant to pathogen infection and partially compensate for disease-related damage. The higher uptake of phosphate has even been proposed as an explanation for the observed biocontrol effect in the case of AMF, as it is known that the plant responses induced by AMF can vary depending on the phosphate availability (Javot et al., 2007). Contrary it could not be demonstrated that the increased supply of phosphate could match the AMF-induced bioprotection. For example, Fritz et al. (2006) reported that mycorrhizal tomato plants had significant less *A. solani* symptoms than non-mycorrhizal plants, although no increase in phosphate uptake was observed. Conversely, when the authors supplied additional phosphate to the mycorrhizal plants, a higher disease severity was even observed. Interestingly, there does not seem to be a direct connection between increased phosphate uptake and plant growth promotion in mycorrhizal plants, since growth depression following AMF colonization has been observed in some cases, even when phosphate transport to the host was active (Smith and Smith, 2011). While phosphate and also nitrogen transfer have been most studied (Tian et al., 2010; Smith et al., 2011), also the importance of AMFs on plant uptake of micro-elements has been investigated. For example, the Zn content in shoots and fruits of field-grown mycorrhizal tomato has been reported to increase compared to tomato mutants with reduced mycorrhizal colonization (rmc) (Cavagnaro et al., 2006).

P. indica can also provide the plant with additional nutrients, probably through solubilization of minerals or by stimulating the plant to take up more nutrients (Peskan-Berghofer et al., 2004) and a growth promoting effect has been observed on a broad range of plants including tomato. Fakhro et al. (2010), for example, reported a strong increase in tomato fruit biomass after colonization with *P. indica* both in soil and hydroponic cultures, mainly due to a larger fruit number than in control plants. The mechanisms of *P. indica* growth promotion are only now starting to be investigated, and a role for several hormones such as ET, GA and cytokinins in growth promotion of *Arabidopsis* has been suggested (Vadassery et al., 2008; Camehl et al., 2010; Jacobs et al., 2011). It has not been investigated whether *P. oligandrum* increases the nutrient content of its host, but it is hypothesized that plant growth promotion, which has been observed in tomato, occurs through the production of the auxin precursor tryptamine (TNH_2) in the rhizosphere (Le Floch et al., 2003). In case of *Trichoderma* spp., increased nutrient uptake as well as plant growth promotion have been observed on many occasions, although this seems to depend on various other factors, including the specific plant and fungal species. For example, Tucci et al. (2011) reported that the growth-promoting effect of *T. harzianum* and *T. atroviride* on tomato shoots and roots depended on the tomato cultivar, with growth responses varying from growth promotion to suppression. Similarly, Alfano et al. (2007) did not observe growth promotion in the tomato–*T. hamatum* T382 interaction, while Chacon et al. (2007) reported a clear tomato growth promotion upon *T. harzianum* CECT 2413 inoculation, with an increase in foliar area and secondary roots. As has been hypothesized for *P. oligandrum*, *Trichoderma* spp. have been reported to produce indole-3-acetic acid (IAA), and a role for auxins has been proposed accordingly for growth promotion in tomato (Gravel et al., 2007; Chowdappa et al., 2013). Gravel et al. (2007) suggested that *T. atroviride* stimulated tomato root growth by balancing the synthesis and degradation of IAA, as well as by limiting ET synthesis through hydrolysis of its precursor 1-aminocyclopropane-1-carboxylic acid (ACC).

Apart from growth promotion, increased root branching is often reported after colonization by AMF (Gamalero et al., 2002; Berta et al., 2005) and *Trichoderma* spp. (Chacon et al., 2007; Tucci et al., 2011), which can also impact plant disease resistance, e.g. positively by increasing its vigour due to elevated nutrient uptake capacity (see above) or negatively by increasing the potential infection surface for pathogens targeting specific infection sites on the roots. Another indirect plant-mediated effect on disease suppression that has been studied only in the case of AMF comprises the altered root exudation of mycorrhizal plants (Hodge, 2000; Jones et al., 2004). Root exudation can vary both quantitatively and qualitatively in mycorrhizal plants, and differences in root exudate composition have been reported, including exudation of amino acids (Harrier and Watson, 2004), flavonoids (Steinkellner et al., 2007), phenolic compounds (McArthur and Knowles, 1992), sugars and amino acids (Sood, 2003; Lioussanne et al., 2008). The altered root exudation is also reflected in the systemic autoregulation of the mycorrhizal colonization by the host (Pinior et al., 1999; Vierheilig et al., 2003): once roots are colonized by AMF, plants appear to be able to regulate further colonization through the release of root exudates. It has been proposed that plants might use this mechanism as a preventative measure against further mycorrhizal colonization and defense against pathogens at the same time (Vierheilig and Piche, 2002; Vierheilig et al., 2008). Root exudates from mycorrhizal tomato plants have been shown to affect several fungal pathogens and even nematodes (Vos et al., 2012a,b), although effects can vary depending on the pathogen and the maturity of the mycorrhizal symbiosis (Scheffknecht et al., 2006). Fillion et al. (1999) showed the reduction of *F. oxysporum* f. sp. chrysanthemi conidia germination when subjected to crude extracts from *G. intraradices* mycelium. On the other hand, a stimulatory effect on the microconidia germination of *F. oxysporum* f. sp. lycopersici was observed by Scheffknecht et al. (2006), and *Phytophthora nicotiana* infection of tomato was not affected by mycorrhizal root exudates (Lioussanne et al., 2009). Lioussanne et al. (2008) also observed that the attraction of *P. nicotianae* zoospores towards mycorrhizal root exudates shifted to repellency, depending on the maturity of the mycorrhizal colonization. Very recently, Hage-Ahmed et al. (2013) observed a significant increase in citrate and chlorogenic acid in the root exudates of mycorrhizal tomato plants under attack of *F. oxysporum* f. sp. lycopersici compared with root exudates of mycorrhizal tomato plants alone. Hence the authors proposed a role for these compounds in the suppression of pathogen germination and subsequent infection of tomato roots.

Changes in the mycorrhizal root exudates composition also lead to alterations in the microbial rhizosphere, where other beneficial micro-organisms reside that can in turn also have a direct or indirect effect on disease suppression. Populations of facultative anaerobic bacteria, fluorescent pseudomonads, *Streptomyces* species and chitinase-producing actinomycetes (Marschner and Baumann, 2003; Wamberg et al., 2003; Harrier and Watson, 2004) have all been shown to be stimulated in the presence of mycorrhizal root exudates. Root exudates originating from mycorrhizal tomato plants have, for example, been reported to attract plant growth promoting bacteria like *P. fluorescens* (Sood, 2003) and to stimulate populations of beneficial soil micro-organisms like *Trichoderma* spp. (Fillion et al., 1999).

4. Conclusions and perspectives

The BCFs discussed above and their possible modes of action are summarized in Table 1. All BCFs are able to colonize tomato roots to some extent. We described their different colonization patterns, ranging from the sophisticated AMF symbiosis to the short-lived *P. oligandrum* root colonization. While AMF, being obligate root symbionts, are obviously very well suited for forming a long-lasting durable symbiosis with their plant host, for *F. oxysporum* and *P. oligandrum*, probably highly resembling their corresponding pathogenic relatives, it is less straightforward to penetrate the plant roots, as shown by the defense response induction in the host plant upon their colonization attempts (Rey et al., 1998; Picard et al., 2000; Benhamou and Garand, 2001; Olivain et al., 2003). Although lacking plant-pathogenic relatives, the colonization pattern of *Trichoderma* spp. resembles that of *F. oxysporum*, with root colonization rarely going beyond the outer cortex due to the reaction of the plant (Yedidia et al., 1999; Chacon et al., 2007). The MTI response of the plant typically involves the formation of structural barriers including the deposition of callose to limit further root colonization. All BCFs have solved this problem in their own specific way, but we are now only starting to unravel the specifics of these interactions. Since AMF are bound to the roots for their survival, it is of primordial importance that they can overcome the MTI response, or succeed in masking their presence. The other BCFs can also survive saprophytically in the soil, thus the need to develop mechanisms for bypassing the plant barriers and colonizing roots more profoundly is probably less crucial in this evolutionary arms race. In recent years the first MAMPs have been identified for *Trichoderma* spp. (Hermosa et al., 2012) and a role for DAMPs is also expected, since these compounds are not only created in plant–pathogen interactions but also by BCFs and can equally lead to the induction of defense responses in the plant.

A certain overlap between MAMPs of BCF and biotrophic pathogens is also anticipated (Zamioudis and Pieterse, 2012). Indeed, beneficial and detrimental plant–fungus interactions have long been investigated separately, and it is only recently that the BCF research community has started to integrate the plant pathological concepts in their domain, due to the increasing amount of indications of common mechanisms for both BCF and pathogens. For example, effector biology has been a major research field in plant–pathogen interactions for some years, while it is only now becoming an area of interest for BCFs as well, with the first AMF effector recently being reported (Kloppholz et al., 2011). The growing convergence of plant–pathogen and plant–BCF research is also facilitated by the shift in scientific attention towards plant roots, where most BCFs naturally reside and which has been long neglected in comparison with above-ground plant parts. This is illustrated by the first report that roots also respond to different MAMPs and set up an MTI response, which was performed only three years ago (Millet et al., 2010).

The general pattern that is often described for ISR-inducing BCFs is that of a weak and transient MTI response of the plant upon colonization, which is followed by a faster and higher induction of the defense responses upon subsequent pathogen attack (Pozo et al., 2010). Priming, in which a central role for JA is generally proposed, has been demonstrated for all BCFs. Showing overlap with biotrophic pathogens, the BCFs are often thought to suppress SA signaling in order to establish their root colonization, while JA signaling is employed by the BCF and functions in the subsequent ISR response (Pozo and Azcon-Aguilar, 2007; Zamioudis and Pieterse, 2012). The picture is certainly more complex, with an intricate interplay of different signaling pathways probably being more realistic, but it is difficult to distill common mechanisms from all experimental data, since defense responses depend for example on fungal and plant species, exact time point of observation and even concentration of BCF inoculum (Mathys et al., 2012; Mukherjee et al., 2012). Research for each BCF also has its own specific focusing points. In order to better understand the general mechanisms of biocontrol, as far as they can be generalized, it would be advisable to streamline future BCF research and focus on the same research questions in all BCFs. In our group we started such more systematic study of tripartite interactions with the plant–BCF–pathogen model *Arabidopsis–T. hamatum* T382–*B. cinerea* (Mathys et al., 2012). Both this BCF and fungal pathogen were chosen because of their broad spectrum of host plants regarding the induction of ISR and plant infection, respectively, including the model plant *Arabidopsis*. First, we performed a genome wide characterization both before and after pathogen infection in order to obtain a more profound insight into the mechanisms of the so-called ISR-prime and boost-phase (Mathys et al., 2012). We discovered a strong similarity between the ISR-prime and SAR, while the JA-pathway appeared more important in the boost phase. In general, we observed significant differences in the plant response induced by either the fungal BCO (ISR) and the fungal pathogen (*Botrytis* induced defense response (BIDR)). Starting from this model tripartite interaction, we then changed one of the interacting partners in order to systematically investigate the tripartite BCF–plant–pathogen interaction and to define to what extent ISR mechanisms can be extrapolated from one experimental system

Table 1
Summary of different biocontrol mechanisms that have been demonstrated for each biocontrol fungus discussed in the review.

BCO	Colonization pattern	Antagonism			Plant-mediated effects		Genome sequenced
		Mycoparasitism	Antibiosis	Competition	ISR	Other plant-mediated effects	
Arbuscular mycorrhizal fungi	Whole root cortex colonized Specialized structures for nutrient transfer Weak and transient plant defense response leads to plant priming	–	–	+	+	+	–
Piriformospora indica	Mainly root maturation zone colonized Biphasic colonization: biotrophic phase followed by cell-death dependent phase	–	–	–	+	+	+
Trichoderma spp.	Dense hyphal network around the root Colonization restricted to outer cortex	+	+	+	+	+	+
Non-pathogenic *Fusarium oxysporum*	Dense hyphal network around the root Colonization rarely reaches outer cortex	–	–	+	+	–	–
Pythium oligandrum	Rapid colonization of all root zones Strong plant defense response limits duration of colonization and the BCO degenerates	+	+	+	+	+	–

to another. Changing the model plant *Arabidopsis* for the crop plant tomato in the tripartite interaction has revealed significant overlap as well as specificities for each interaction. For example, the flavonoid and JA-biosynthesis pathways were found induced in both systems, while induction of the SA-signaling pathway appeared to be plant-specific (Yang et al., unpublished). Using this systematic approach, we recently also changed the model BCF T382 and thereby identified several ISR markers genes that seemed preserved by different *Trichoderma* strains and can assist us in future screenings of potential ISR-inducing BCFs (De Cremer et al., 2013; Vos et al., unpublished).

Currently global genome-wide studies are still missing that might improve our insight into both plant–pathogen, plant–BCF and tripartite interactions. With the emergence of next generation sequencing techniques like RNA sequencing, however, this is expected to change rapidly. The technique is already being adopted for two-partite studies focusing on plant–pathogen (Kawahara et al., 2012; Kyndt et al., 2012) or plant–BCF interactions (Tanaka et al., 2012), and the first next-generation studies of tripartite interactions are emerging (Perazzolli et al., 2012). These experiments are facilitated by the increasing amount of available sequenced genomes of all players in the tripartite interactions. Some of the most important tomato plant pathogens that have already been sequenced include *B. cinerea* (Amselem et al., 2011), *P. infestans* (Haas et al., 2009), *F. oxysporum* f. sp. lycopersici (Ma et al., 2010) and *Pythium ultimum* (Lévesque et al., 2010). Concerning the BCFs, so far only the genomes of *P. indica* (Zuccaro et al., 2011) and of the *Trichoderma* spp. *T. atroviride*, *T. virens* and *T. reesei* (Kubicek et al., 2011) have been sequenced and additional projects for *T. harzianum* and *T. asperellum* are underway (Druzhinina et al., 2011).

This information forms the starting point for a great leap forward in our understanding of the molecular basis of the tripartite interactions. It will also help us to further elucidate the parallels that are emerging between BCFs and pathogenic fungi, which will certainly be a major focus in the years to come. The detailed molecular data will also enable us to improve the use of these BCFs in agriculture, for example by identification of novel potential strains from fungal libraries, by targeted genetic improvement of the known BCF strains or the identification and isolation of their bioactive compounds. Another important issue to address in view of future practical applications is the combination of different BCFs and the mechanisms underlying their interplay. Anith et al. (2011), for example, investigated the effect of combining *T. harzianum* and *P. indica*. The *Trichoderma* strain turned out to antagonize *P. indica* growth as well as subsequent root colonization, however inoculation of pepper plants with both BCFs resulted in higher plant weight compared to single inoculations. For the combination of AMF and *Trichoderma* spp., different outcomes have been noted. Some studies suggested a synergism between both BCFs, while others observed an antagonistic effect of *Trichoderma* spp. on AMF or vice versa (McAllister et al., 1994; Green et al., 1999). In addition, the combination of BCFs with bioactive compounds is also a promising topic that merits further investigation. These compounds could for example be derived from known *Trichoderma* spp. or *P. oligandrum* MAMPs (Vinale et al., 2009; Benhamou et al., 2012).

In conclusion, it is clearly an exciting era for the study of biocontrol tripartite interactions. So far we have undoubtedly only uncovered the tip of the iceberg, but with the emergence of next-generation sequencing tools and an increasing amount of available sequenced genomes together with the tighter collaboration between plant pathologists and BCF scientists, our knowledge on the tripartite interactions is expected to rise steeply in the years ahead.

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